Calculate the percent particles in each group from the total number measured. Determine the percent relative weight for each group as follows:

Plate type particles. Relative weight= (ratio)^2×% of total particles in group.

$$\%\,\text{relative weight} = \frac{\text{Relative weight} \times 100}{\text{Total relative weight}}$$

Rod-shaped particles. In the case of rod-shaped particles measure the width as well as the length.

Relative weight=ratio × average width × % of total particles in group

$$\%\,\text{relative weight} = \frac{\text{Relative weight} \times 100}{\text{Total relative weight}}$$

When examined by the method described in this section not less than 50 percent of the total relative weight of the penicillin in the drug consists of penicillin having a particle size of not less than 50 microns in length.

§ 436.503 Procaine penicillin and buffered crystalline penicillin for aqueous injection.

(a) *Total potency* (*except in single-dose container*), *sterility, moisture, pyrogens, toxicity, pH.* Proceed as directed in § 440.274b(b) of this chapter.

(b) *Buffered crystalline penicillin content*—(1) *Preparation of the solution for assay.* Add the indicated amount of distilled water to the contents of a vial of the sample, and shake well. Withdraw one dose of the suspension with a hypodermic syringe and place in a 10-milliliter volumetric flask. Add 20-percent sodium sulfate solution almost to the mark, centrifuge sufficiently to see the meniscus, make to volume with 20-percent sodium sulfate solution, shake well, and centrifuge to obtain a clear or reasonably clear solution. Dilute a 5.0-milliliter aliquot of this clear solution with 1-percent phosphate buffer, pH 6.0, to give a solution for assay of approximately 2,000 units per milliliter.

(2) *Iodometric assay for total penicillin in the solution for assay.* Determine the quantity of penicillin in the solution for assay by the iodometric assay procedure described in § 440.80a(b)(5)(iv)(*a*) of this chapter.

(3) *Colorimetric determination of procaine penicillin in the solution for assay.* Transfer an aliquot of the solution for assay to a 50-milliliter volumetric flask. Determine the quantity of procaine penicillin in this solution by the following method:

(i) *Reagents*—(*a*) *Sodium nitrite solution.* Dissolve 0.1 gram of sodium nitrite in 100 milliliters of distilled water. Prepare fresh solution every week and store under refrigeration.

(*b*) *Ammonium sulfamate solution.* Dissolve 0.5 gram of ammonium sulfamate in 100 milliliters of distilled water and store under refrigeration.

(*c*) *N-(1-naphthyl)-ethylenediamine solution.* Dissolve 0.1 gram of *N*-(1-naphthyl) ethylenediamine dihydrochloride in 100 milliliters of distilled water. Prepare fresh solutions every week and store under refrigeration.

(*d*) *Standard procaine solution.* Prepare a standard solution containing 27.55 milligrams of procaine hydrochloride U.S.P. in a liter of distilled water (each milliliter of the standard solution is equivalent to 60 units of procaine penicillin).

(ii) *Standards.* Transfer, respectively, 1.0, 2.0, 3.0, 4.0, and 5.0 milliliters of the standard solution and 5.0 milliliters of distilled water to each of six 50-milliliter volumetric flasks. Add 4.0, 3.0, 2.0, and 1.0 milliliters of water to the first four flasks, respectively, to give each a volume to 5.0 milliliters.

(iii) *Procedure.* To each flask for the standards and the solution for assay add 0.5 milliliter of 4 *N* HCl, 1.0 milliliter of the sodium nitrite solution, 1.0 milliliter of the ammonium sulfamate, and 1.0 milliliter of the *N*-(1-naphthyl)-ethylenediamine solution. Mix and wait two minutes after each addition. Make each flask to volume of 50 milliliters with distilled water. Determine the absorbency of the colored solutions at 550 Mμ in a suitable photo electric colorimeter. The instrument is balanced so that the zero concentration reads zero absorbency. Plot the standard curve on coordinate graph paper. Obtain the procaine penicillin content of the solution for assay directly from the point on the standard curve corresponding to its absorbency.

(4) The content of buffered crystalline pencillin in one dose of the product is calculated as follows:

$$A=(B-C)F,$$

where:

A=buffered crystalline penicillin content of the product.
B=total number of units of penicillin per milliliter as determined in paragraph (b)(2) of this section.
C=number of units of procaine penicillin per milliliter as determined in paragaph (b)(3) of this section.
F=appropriate dilution factor depending on the dilution made in the preparation of the solution for assay.

The content of buffered crystalline penicillin in the batch is satisfactory when determined by the method described in this paragraph if it is not less than 85 percent of that which it is represented to contain.

(c) *Procaine penicillin.* The procaine penicillin content of the batch is the difference between the total potency determined by the method described in paragraph (a) or (d) of this section and the content of the buffered crystalline penicillin determined by the method described in paragraph (b) of this section. The procaine penicillin content of the batch is satisfactory when determined by the method described in this paragraph if it is not less than 85 percent of that which it is represented to contain.

(d) *Total potency of a one-dose container.* Wash out the material remaining in the 10-milliliter volumetric flask referred to in paragraph (b)(1) of this section with 1-percent phosphate buffer, pH 6.0. Dilute to give a concentration of approximately 2,000 units per milliliter, and assay by the iodometric method described in § 440.80a (b)(5)(iv)(*a*) of this chapter. Obtain the total potency by adding the number of units found in this solution (units per milliliter × volume) to the number of units found (units per milliliter × volume) in the solution assayed in accordance with paragraph (b)(2) of this section.

§ 436.504 Penicillin-bacitracin ointment.

(a) *Potency*—(1) *Penicillin content.* Proceed as directed in § 540.380a(b)(1) of this chapter, except the last sentence of that paragraph. Its content of penicillin is satisfactory if it contains not less than 85 percent of the number of units it is represented to contain.

(2) *Bacitracin content.* Proceed as directed in § 448.510a(b)(1) of this chapter, except that sufficient penicillinase is added to the sample under test to completely inactivate the penicillin present. Its content of bacitracin is satisfactory if it contains not less than 85 percent of the number of units it is represented to contain.

(b) *Moisture.* Proceed as directed in § 436.201.

[39 FR 18944, May 30, 1974, as amended at 40 FR 13497, Mar. 27, 1975]

§ 436.505 Penicillin-streptomycin-bacitracin ointment; penicillin-dihydrostreptomycin-bacitracin ointment; penicillin-streptomycin-bacitracin methylene disalicylate ointment; penicillin-dihydrostreptomycin-bacitracin methylene disalicylate ointment.

(a) *Potency*—(1) *Content of penicillin, streptomycin, and dihydrostreptomycin.* Proceed as directed in § 536.501(a) of this chapter.

(2) *Bacitracin content.* Proceed as directed in § 448.510a(b)(1) of this chapter, except that:

(i) Sufficient penicillinase is added to the sample under test to completely inactivate the penicillin present.

(ii) Use as the test organism the streptomycin dihydrostreptomycin resistant strain of either Micrococcus flavus (ATCC 10240A)[1] or Sarcina subflava (ATCC 7468/d),[1] grown and maintained in media containing 500 micrograms of streptomycin or dihydrostreptomycin per milliliter of media, or calculate from the quantity of streptomycin or dihydrostreptomycin found, using the method prescribed by paragraph (a)(1) of this section, the quantity that would be present when the sample is diluted to contain one unit of bacitracin (labeled potency) per milliliter. Prepare the bacitracin standard curve by adding the calculated quantity of streptomycin or dihydrostreptomycin to each concentration of bacitracin used for

[1] Available from: American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852.